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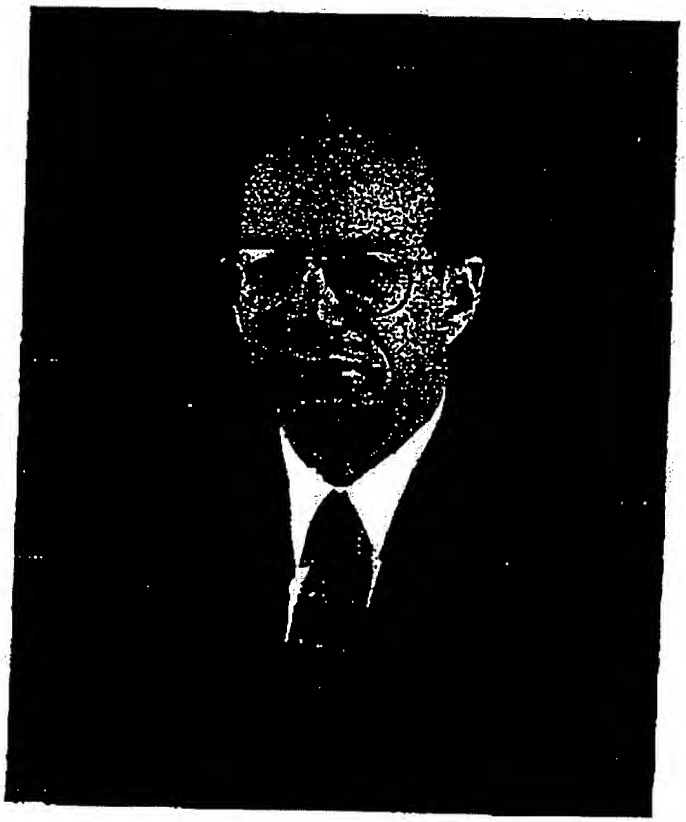
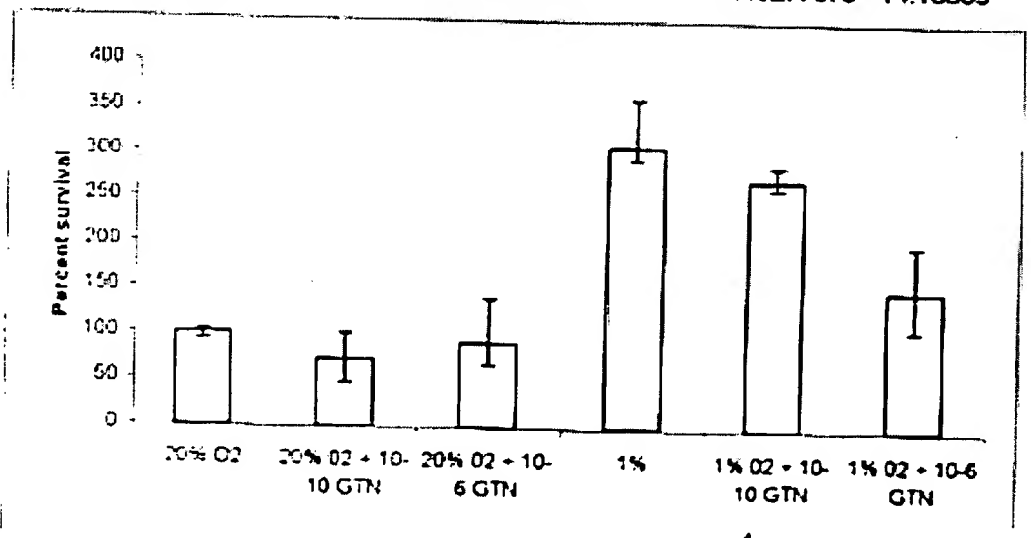


Norm to 20%	20% O2	20% 02 + 10-6 GTN	20% 02 + 10-10 GTN	1%	1% 02 + 10-6 GTN	1% 02 + 10-10 GTN
	0.780488	1.510125101	1.156691567	2.902439	1.078429179	2.88740048
	1	1.47799478	1.028170282	3.065497	0.978574625	2.977631745
	1.02439	1.124561246	0.931779318	2.902439	1.09840009	2.075319095
	0.934426	0.683619995	0.503146956	3.64708	2.184494373	2.636490047
	1.065574	0.665143779	0.462895199	3.606557	2.034578093	2.616364169
		0.665143779	0.442769321	3.606557	1.906078424	2.797497073
				3.079757		
				2.937926		
				3.100018		
Median	1	0.90409062	0.717463137	3.079757	1.502239257	2.71699356
1q	0.934426	0.669762833	0.472958138	2.937926	1.083421906	2.621395638
3q	1.02439	1.389636396	1.004072541	3.606557	2.002453175	2.864924628
plus	0.02439	0.485545776	0.286609404	0.526801	0.500213919	0.147931069
minus	0.065574	0.234327788	0.244504998	0.141831	0.41881735	0.095597922
percent	78.04878	151.0125101	115.6691567	290.2439	107.8429179	288.740048
	100	147.799478	102.8170282	306.5497	97.85746252	297.7631745
	102.439	112.4561246	93.17793178	290.2439	109.840009	207.5319095
	93.44262	68.36199946	50.31469555	364.708	218.4494373	263.6490047
	106.5574	66.51437786	46.28951991	360.6557	203.4578093	261.6364169
		66.51437786	44.27693208	360.6557	190.6078424	279.7497073
				307.9757		
				293.7926		
				310.0018		

	20% O2	20% 02 + 10-6 GTN	20% 02 + 10-10 GTN	1%	1% 02 + 10-6 GTN	1% 02 + 10-10 GTN
Median	100	90.40906201	71.74631366	307.9757	150.2239257	271.699356
1q	93.44262	66.97628326	47.29581382	293.7926	108.3421906	262.1395638
3q	102.439	138.9636396	100.4072541	360.6557	200.2453175	286.4924628
plus	2.439024	48.55457762	28.66094041	52.68005	50.02139189	14.79310686
minus	6.557377	23.43277875	24.45049985	14.18309	41.88173502	9.559792155

	20% O2	20% 02 + 10-10 GTN	20% 02 + 10-6 GTN	1%	1% 02 + 10-10 GTN	1% 02 + 10-6 GTN
Median	100	71.74631366	90.40906201	307.9757	271.699356	150.2239257
1q	93.44262	47.29581382	66.97628326	293.7926	262.1395638	108.3421906
3q	102.439	100.4072541	138.9636396	360.6557	286.4924628	200.2453175
plus	2.439024	28.66094041	48.55457762	52.68005	14.79310686	50.02139189
minus	6.557377	24.45049985	23.43277875	14.18309	9.559792155	41.88173502



36
0.173913043

20% O_2, 25μm, 10^{-10} GTN

189
7

hypoxic, 0μm, 0 GTN

103
7

hypoxic, 25μm, 0 GTN

127
4

hypoxic, 0μm, 10^{-6} GTN

127
4

hypoxic, 25μm, 10^{-6} GTN

90
4

hypoxic 0μm, 10^{-10} GTN

143
4

hypoxic 25μm, 10^{-10} GTN

108
4

data #	[O_2]	[adr]	[GTN]	[cell]
#1	20% O_2	0	0	5×10^2
	1%	0	0	5×10^2
#2	20	25	0	
	1	25	0	
#3	20	0	10^{-6}	
	1	0	10^{-6}	
			10^{-6}	
			10^{-6}	

Set up MDA-MB-231 cells for Experiment 19990917 - Adr - hypoxia - colony assay
- grow in RPMI + 5% Fbs + P/S

- cell density for experiment looked good
- changed media on plates - 150mm received 15ml
- 100mm received 7ml
placed in 20% O2 incubator and hypoxic chamber (11:20)

adriamycin dilutions - stock conc. 2mg/ml
1μm adm : 0.580 ug/ml mw = 580
0.58 ug/ml = 0.29μl in 1ml : 29 x 25ml = 7.25μl
7.25μl of adm into 25ml
25μm adm : 14.5μg/ml
: 362.5μl of adm in 50ml
50μm adm : 29μg/ml
: 725μl of adm in 50ml
100μm adm = 58ug/ml
: 1450μl of adm in 50ml
- make up in RPMI + 5% Fbs + P/S
- remove plates of cells from chamber - 1% O2
(monitor reading)
removed media from plates and added fresh media to 0μm adm controls and adriamycin/media to appropriate plates.

For other drugs: [ ] from lit.

cyclophosphamide 12.5 μM → 500 μM 0–100 μM
(Kobayashi et al, 1993)

methotrexate
↳ 25, 50, 100, 150, 200 μM

cisplatin? 0–14 μg/mL

Counting: 1/10 expt.

Plate	[dox]	[O2]	# cells	# colonies	P.E.	SF.
#15	25 μM	20%	5×10^4	2	4×10^{-5}	6.93×10^{-5}
				1	2×10^{-5}	$3.\times10^{-5}$
				2	1×10^{-5}	6.73×10^{-5}
		1%	5×10^4	45	9×10^{-4}	2.21×10^{-3}
				24	4.8×10^{-4}	0.0018
				40	8×10^{-4}	0.00197

Next expt.

	0 μM	25 μM
SNP	+ 5×10^3, 10^3, 5×10^2, 10^2	+ 5×10^4, 10^4, 5×10^3, 10^3
GTN	+	+
Control	−	−

1% 20%

Everything x2 (One hypoxic, one normoxic)
∴ 12 100mm plates.

③ 30mg in 10mL : 10^{-2} M

Not enough plates (!).
So: Set-up.

②

[dox]	1% O_2 0	1% O_2 25	20% O_2 0	20% O_2 25
CONTROL	✓	✓	✓	✓
SNP 10^{-6}	✓	✓	—	—
10^{-10}	✓	✓	—	—
GTN 10^{-6}	✓	✓	—	—
10^{-10}	✓	✓	—	—

① Put 2 normox plates in @ 37°C @ 11.15am
② Put plates in hypoxia @ 11.35am

④ Remove media.
Add 7mL of either RPMI + P/S
or RPMI + P/S + approp [ ] of GTN or SNP.

CHECK PLATES FROM
on 0μM : colonies growing (v. small still)
: stain 5x10^3 ¢ (will be confluent soon).
1μM : stain 5x10^3 ¢

* All media w/ adriamycin has to be treated w/ charcoal (so don't put into bleach)

Experiment w/ NO donor: Expt

① took out of hypoxic chamber @ 11am
37°C incubator

② Removed media and added 7mL of
a) RPMI + FBS + P/S
or b) RPMI + FBS + P/S + adriamycin
↳ 25μM conc.

put 250μM in incubator @ 11:15am
0μM in incubator @ 11:20am.

⇒ 37°C, 20% O_2 for 1 hr.
↳ take out @ 12:15 pm

③ Remove media from all plates *
- add 2mL trypsin/EDTA
- oil for ~5min

③ Add 8mL of media (RPMI + FBS + P/S)
- resuspend &
- put into 50mL conicals
- count.

$[O_2]$	[Adx]	[GTN]	count	#/mL ($\# \times 10^4 \times 2$)
20% O_2	0	0	94/4	47×10^4
20% O_2	25	0	193/4	96.5×10^4
20%	0	10^{-6}	259/4	129.5×10^4
20%	25	10^{-6}	184/4	92×10^4
20%	0	10^{-10}	171/4	85.5×10^4
20%	25	10^{-10}	189/4	94.5×10^4

experiment for Thursday

	0µM	25µM
SNP	+ 5x10^3	+ 5x10^4 ✓
	10^3	10^4 ✓
	5x10^2 ✓	5x10^3
	10^2 ✓	10^3
GTN	+	+
⊖	−	−

plates - set up 6x2 = 12 100mm plates

make up 50ml of adriamycin (25µM)
need 24, 6 well plates
- Thursday exp cancelled - O_2 conc 16.4% - try again Thursday

		0µm	25µm
10^{-6}	GTN Conc. 1	5x10^2	5x10^4
		10^2	10^4
10^{-10}	GTN Conc. 2	5x10^2	5x10^4
		10^2	10^4
	−	5x10^2	5x10^4
		10^2	10^4

6x2
12 plates

adrenal p/s
- subculture cells
- add up 12 plates for Monday
- stained remaining plates from experiment

plate #	µM	cell #
4	100	5×10^4
3	100	10^3
9	50	10^5
10	50	5×10^4

checked plates from adr-hyp.
- experiment - adrg_hyp_GTN
- cell counts

20% O_2, 0µm, 0 GTN

$\frac{94}{4/2} \times 10^4 \times 2 = 47 \times 10^4$ /ml

20% O_2, 25µm, 0 GTN

$\frac{193}{4}$

20% O_2, 0µm, 10^{-6} GTN

$\frac{259}{4}$

20% O_2 25µM, 10^{-6} GTN

$\frac{184}{4}$

20% O_2 0µm, 10^{-10} GTN

$\frac{171}{4}$

The Effects of Nitric Oxide on the Urokinase System of Plasminogen Activation and on Cellular Invasiveness

425 Thesis

By: Lynne Postovit

Supervisor: Dr. C. Graham

ABSTRACT

Cellular invasion is a process which characterizes such biological occurrences as tumour progression and zygote implantation. It also plays a critical role in the remodelling of the uterine vasculature during the first trimester of pregnancy. Failure of trophoblast cells to complete this function may lead to such pathologies as pre-eclampsia. Both tumour progression and uterine remodelling have been shown to occur under low oxygen levels. The urokinase system of plasminogen activation plays a vital role in cellular invasiveness. Further, the expression of the urokinase-type plasminogen activator receptor (uPAR), as well as *in vitro* cellular invasion, increase under hypoxic conditions. In this study, immortalized human trophoblasts (HTR-8/SVneo cells) were used to study the effects of low levels of nitric oxide (NO) on uPAR expression and cellular invasion under hypoxic conditions. Exposure to the NO donors, sodium nitroprusside (SNP) and glyceryl trinitrate (GTN) resulted in a decrease in uPAR mRNA levels, as determined by Northern blot analysis. Further, they also decreased the ability of trophoblast cells to invade an extracellular matrix *in vitro*. These findings indicate that under hypoxic conditions, low levels of NO inhibit uPAR expression as well as *in vitro* cellular invasiveness. Through a similar mechanism, NO donors may be used therapeutically to inhibit tumour progression *in vivo*. Further, by inhibiting the invasiveness of trophoblast cells during the first trimester of gestation, NO may be playing an etiological role in the development of pre-eclampsia.

INTRODUCTION

Pre-eclampsia is a common pathology of human pregnancy, causing such problems as